

EXHIBIT B

Tolerance of Volunteers to Cyclosporine A-dilauroylphosphatidylcholine Liposome Aerosol

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Cyclosporine A (CsA) in liposomes of dilauroylphosphatidylcholine (DLPC), containing 118 μg of CsA/L of aerosol with a particle size of 1.6 to 1.7 μm diameter, was inhaled by 10 nonsmoking, normal volunteers each for 45 min. Aerosol was administered through an Aerotech II nebulizer (CIS-US, Inc., Bedford, MA) mouthpiece. Eight of the 10 volunteers had tracheal irritation and intermittent coughing following exposure. FEV₁ and FVC values were mildly reduced, but returned to normal in 1 h. Blood chemical and hematologic values were unchanged at any time point after as opposed to before inhalation. Nine of the 10 volunteers later inhaled DLPC only, administered through the nebulizer mouthpiece. There was no change in FEV₁ or FVC values, and there was no coughing or tracheal irritation. Subsequently, five of the volunteers who had previously had respiratory reactions inhaled CsA-DLPC liposome aerosol for 45-min, but through a mouth-only face mask. There was no tracheal irritation, coughing, or changes in spirometric measures. Blood concentrations of CsA at 15 min after the 45-min inhalation with a face mask averaged 83 ± 42 ng/ml (mean \pm SD). At 24 h after treatment, CsA was undetectable in blood of the initial 10 volunteers. These studies indicate that CsA-DLPC liposome aerosol can be safely explored as a treatment for patients with moderately severe asthma. **Gilbert BE, Knight C, Alvarez FG, Waldrep JC, Rodarte JR, Knight V, Eschenbacher WL. Tolerance of volunteers to cyclosporine A-dilauroylphosphatidylcholine liposome aerosol.**

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The treatment of asthma with cyclosporine A (CsA) given orally has been reported by several investigators. Nizankowska and colleagues (1) described improvement in a patient with aspirin-induced asthma. During a several-month period of treatment, the patient's pulmonary function improved and it was possible to reduce the dose of prednisone. Szczeklik and coworkers (2), at the same clinic as Nizankowska and colleagues, reported similar improvement in six patients with longstanding asthma, and a reduction in their requirement for prednisone, but six other patients did not respond. In a more detailed study of 34 patients with steroid-resistant chronic asthma, these investigators noted only limited benefit (3). Klein and associates (4) observed a marked reduction in severity of asthma and reduced requirement for glucocorticoids in three patients given CsA by mouth. The foregoing studies used low oral doses of CsA, aimed at keeping blood trough levels of drug in the range of 70 to 150 ng/ml. Oral doses of up to 3.5 mg/kg/d were required to produce these levels. Fifty patients were involved in these studies, of whom 10 (20%) were considered to have improvement. Treatment of one patient was discontinued because of hypertension.

Alexander and colleagues (5) found that oral CsA treat-

ment at 5 mg/kg/d in a crossover study of 33 patients increased morning peak expiratory flows, with a reduction in exacerbations of asthma. Lock and coworkers (6), at the same clinic as Alexander and colleagues and using the same dose of CsA, found similar favorable results. In both of the preceding studies there were some patients who did not respond to treatment, and there was hypertension, hypertrichosis, gum hypertrophy, and other signs of intolerance to the drug. Mean values for creatinine and urea increased significantly in latter study by Lock and coworkers.

The first group of patients, who received the lowest doses of CsA, showed a limited response to treatment and very little toxicity, whereas the second group, which received the higher dose, showed more favorable responses to treatment, but also had some definite findings of toxicity.

A possible explanation for these variable responses to oral CsA treatment is contained in the work of Martinet and associates (7). They found that oral administration of CsA in high dosage did not benefit eight subjects with sarcoid. They further noted that mononuclear cells obtained from the subjects' lungs following 6 mo of treatment demonstrated the same degree of spontaneous release of interleukin-2 (IL-2) and monocyte chemoattractant factor (MCF) *in vitro* as did cells obtained before treatment. Martinet and associates did, however, find that lung T-cells from sarcoid patients, when tested with CsA *in vitro*, showed almost complete suppression of the response to IL-2 and MCF. They also found that although trough levels of CsA averaged 220 ng/ml in blood, bronchoalveolar wash fluids contained undetectable amounts of CsA. It thus appeared that oral administration of CsA did not produce suffi-

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cient concentrations of CsA in the lungs to provide effective therapy.

Targeted delivery of CsA directly to the lungs by aerosol should increase the therapeutic availability of CsA for localized immunosuppression, which could in turn reduce the incidence of CsA-associated toxicity. We have previously reported the suitability of CsA-liposome aerosols for the distribution of CsA throughout the respiratory tract (8, 9), and the lack of acute toxicity of such treatment in rats (10). The study reported here extends these studies to the first clinical trial of a CsA-dilauroylphosphatidylcholine (DLPC) liposome formulation for aerosol administration in humans.

METHODS

Description of Subjects

Ten nonsmoking normal, healthy volunteers (three female and seven male) between the ages of 22 and 54 yr were enrolled in the study (Table 1). Each subject was found to be healthy on the basis of history, physical examination, and spirometry. Female subjects were surgically sterile. Nine of the initial 10 subjects participated in Part 2 of the study, and five of the 10 participated in Part 3 of the study. Protocols were approved by the Baylor Affiliates Review Board, Baylor College of Medicine, and informed written consent was obtained from the volunteers.

Screening Procedures

Before the start of the study, a medical history was obtained and physical examination performed on each subject. Blood chemistry values including electrolytes; renal and liver function tests; and cholesterol; complete blood count (CBC) with differential count; prothrombin time (PT), partial thromboplastin time (PTT); urinalysis; and CsA levels were obtained. Spirometry was done with the Sensormedics Model 922 Automated Spirometer (Yorba Linda, CA) according to American Thoracic Society (ATS) guidelines (11). Predicted values for spirometry were taken from Crapo and colleagues (12).

Study Procedures: Part 1

Each subject received a continuous 45-min treatment with aerosolized CsA-DLPC (1:7.5, wt/wt) liposomes administered through the mouthpiece of an Aerotech II jet nebulizer (CIS-US Inc., Bedford, MA) op-

erated at 10 L/min. Particle size of the aerosol at the tip of the mouthpiece was 1.6 μ m mass median aerodynamic diameter (MMAD), with a geometric standard deviation (GSD) of 2.4 as measured with an Andersen cascade impactor (Andersen Instruments Inc., Atlanta, GA) (10). Aerosol, generated from an aqueous suspension of liposomes, contained 113 μ g of CsA/L. During exposure to aerosol, each subject was in the seated position in an aerosol collection chamber (Lone Star Medical Industries, Houston, TX), which was equipped with a high-efficiency particulate air (HEPA) filter, and air was evacuated continuously during and after aerosol exposures.

Spirometry was repeated immediately after aerosol exposure and at 1, 6, and 24 h and 1 wk later. Laboratory tests were repeated at 24 h and at 1 wk later. CsA blood levels were not measured immediately, as in Part 3 (see below), but were measured 24 h after exposure. Adverse events were followed to resolution.

Study Procedures: Part 2

Based on the intermittent coughing noted during the initial evaluations of the aerosol treatment, a second aerosol experiment was conducted (Part 2) in which DLPC liposomes only were administered. All of the original 10 subjects were invited to return for Part 2 of the study. Nine of the 10 returned. The 10th subject had scheduling conflicts and elected not to participate. Each subject received a continuous 45-min treatment of aerosolized DLPC liposomes administered through the mouthpiece of an Aerotech II nebulizer, with all conditions being the same as in Part 1. Spirometry was done before the treatment, and was repeated immediately after and at 1 h and 6 h. No laboratory tests were performed. Adverse events were followed to resolution.

Study Procedures: Part 3

To evaluate the possible role of CsA and/or the mouthpiece of the Aerotech II nebulizer in the intermittent coughing noted in Part 1 of the study, five of the 10 volunteers from Part 1, who had experienced coughing, were reevaluated with a mouth-only face mask (Hans Rudolph Inc., Kansas City, MO) for the administration of CsA-DLPC liposomes. At the junction of the face mask with the aerosol input tube, a sidearm with a 24-in, 1-cm I.D. accordion tube, open at the end, was attached (13). This tube filled with aerosol during patients' exhalation, and provided aerosol for the subjects during periods of peak inspiration. Some aerosol was visible escaping from the open-ended tube during subjects' exhalations. Each subject received a continuous

TABLE 1

DESCRIPTION AND CYCLOSPORIN A LEVELS IN THE BLOOD OF VOLUNTEERS IN THE CYCLOSPORINE A-DILAULOYLPHOSPHATIDYLCHOLINE LIPOSOME AEROSOL TOLERANCE STUDY*

	Administered by Mouthpiece		Administered by Face Mask
	Part 1: CsA-DLPC	Part 2: DLPC Only	Part 3: CsA-DLPC
Number exposed	10	9	5
Female	3	3	1
Male	7	6	4
Age, yr			
Mean \pm SD	34.6 \pm 9.2	36.0 \pm 8.6	35.3 \pm 6.6
Range	22.3–54.1	24.1–54.2	24.2–40.5
Body weight, kg			
Mean \pm SD	76.8 \pm 17.9	78.0 \pm 18.6	74.1 \pm 12.3
Range	56.8–118.2	56.8–118.2	61.8–93.2
CsA blood level at 15 min post aerosol, ng/ml			
Mean \pm SD			83 \pm 42
Range			25–140
CsA blood level 24 h post aerosol, ng/ml			
Mean	0 [†]		

Definition of abbreviations: CsA = cyclosporine A; DLPC = dilauroylphosphatidylcholine.

* The study was conducted in three parts: (1) initially in 10 volunteers, CsA-DLPC liposome aerosol was given through a mouthpiece attached to an Aerotech II nebulizer; (2) subsequently, in nine of the original 10 volunteers, DLPC-only liposome aerosol was also given through the mouthpiece; and (3) in a subset of five of the 10 original volunteers, CsA-DLPC aerosol was given through a mouth-only face mask (see Methods).

[†] Minimum detection level for CsA in blood was 25 ng/ml.

45-min treatment of aerosolized CsA-DLPC liposomes administered from an Aerotech II nebulizer attached by corrugated tubing to the mouth-only face mask. Particle size of the aerosol in the face mask was 1.7 μ m MMAD, with a GSD of 1.9, and the aerosol contained 122 μ g of CsA/L. These values are very similar to those in Part 1 of the study. All other conditions were as in Parts 1 and 2. Spirometry was done before the treatment and repeated immediately afterward and at 1 h. CsA blood levels were obtained 15 min after the end of the 45-min treatment. Adverse events were followed to resolution.

CsA and DLPC Liposomes

CsA-DLPC liposomes were prepared by lyophilization of a mixture of CsA and DLPC (1:7.5, wt/wt) dissolved in *t*-butanol (Fisher Scientific, Houston, TX) by Pharmacia Upjohn, Inc. (Albuquerque, NM) under contract. The liposomal drug was stored as a lyophilized powder at -20° C. Before use, 5 ml of sterile, endotoxin-free water for injection was added to each of three vials, and the reconstituted liposome preparations were shaken for 20 to 30 min at room temperature. The final concentrations were 5.5 to 6 mg of CsA/ml. The entire 5 ml of drug added to the nebulizer was sufficient for 15 min of aerosolization. After 15 min, new drug and nebulizer were used.

Liposomes consisting of DLPC only were prepared by Avanti Polar Lipids (Alabaster, AL) as previously described (14), and were reconstituted to provide 37.5 mg of DLPC/ml H₂O.

Blood Chemistry, Hematology, and CsA Levels

Blood chemistry analyses, as described earlier for the screening procedures, and CsA blood levels, were determined by the Department of Pathology of St. Luke's Episcopal Hospital, Houston, TX. The minimum CsA detection level with the high-pressure liquid chromatography (HPLC) methodology used was 25 ng/ml of blood.

Regional CsA Dose Calculations

Dosage calculations were based on the report of Phalen (15) that the average ventilation requirement for human adults is 0.108 L·min/kg of body weight, whereas the regional deposition of CsA was based on a lung model described by Persons and colleagues (16). Persons and colleagues' model assumed a V_T of 1,000 ml, functional residual volume of 3,300 ml, respiratory rate of 2 s in and 2 s out, no pause, breathing by mouth, and a hygroscopicity equivalent to half-normal saline (0.0045 gm/ml) with a density of 1.0 gm/ml. The measured particle size was 1.8 μ m MMAD, with a GSD of 2.0.

Statistical Analyses

Data analyses were performed with the True Epistat statistical package from Epistat Services, Richardson, TX. *p* Values (two-tailed) were based on analyses of data with Student's *t* test or the matched *t* test.

RESULTS

Exposure to CsA-DLPC Aerosol (Part 1)

Groups of volunteers were exposed to liposome aerosols on three occasions (Table 1). In the first experiment, 10 volunteers were given 45-min inhalations of CsA-DLPC liposome aerosol. In two succeeding experiments, subsets of the 10 volunteers were given 45-min exposures as indicated in Table 1. Spirometry (see METHODS) was performed throughout.

Table 2 shows the spirometric FEV₁ values recorded at the start of aerosol exposure and the percent change in these values at the end of aerosol exposure in the first experimental group of 10 volunteers given CsA-DLPC liposomes by mouthpiece. The mean and individual FEV₁ and FVC values decreased slightly from the start of aerosol exposure to immediately following it. This slight depression immediately after exposure (-7.9%) was statistically significant for both FEV₁ (Table 2) and FVC (not shown) ($p = 0.015$ and $p = 0.041$, respectively; matched *t* test, two-tailed). Values at 6 h and 24 h and at 1 wk were almost identical to those at 1 h after exposure. Intermittent coughing and associated tracheal irritation of mild to moderate severity were experienced by eight of the 10 volunteers (Table 2), sometimes beginning before the end of the 45-min exposure to CsA-DLPC.

Exposure to DLPC-only Aerosol (Part 2)

A few days after the end of the first study, nine of the volunteers were given 45-min inhalations by mouthpiece of an aerosol consisting of liposomes containing DLPC only (i.e., vehicle control) (Tables 1 and 2). FEV₁ (Table 2) and FVC (not shown) values were unchanged before and after inhalations (-1.1% ; Table 2). The change in FEV₁ was statistically significantly dif-

TABLE 2
FEV₁ VALUES AND SYMPTOMS OF SUBJECTS ADMINISTERED CYCLOSPORINE A-DILAUYOLPHOSPHATIDYLCHOLINE LIPOSOME AEROSOL*

Subject	Administered by Mouthpiece				Administered by Face Mask			
	Part 1: CsA-DLPC	Part 2: DLPC-Only			Part 3: CsA-DLPC			
	Start	Change (%) ¹	I/C ²	Time ³ (min)	Start	Change (%) ¹	I/C ²	Time ³ (min)
1	4.09	-5.1	Yes	120	3.98	-2.8	No	0
2	5.14	-1.8	No	0	5.05	-0.6	No	0
3	5.06	-13.4	Yes	90	4.82	0.6	No	4.83
4	4.38	-3.2	Yes	20	4.27	1.2	No	4.28
5	4.77	-10.1	Yes	360	4.76	-5.3	No	0
6	3.67	-30.5	Yes	120	3.66	-1.4	No	3.54
7	2.81	-4.6	Yes	3	2.72	0.4	No	0
8	4.42	-7.9	Yes	240	4.2	0.2	No	0
9	3.14	-2.5	Yes	45	3.14	-2.2	No	3.27
10	3.04	0	No	0	2.93	0.3	No	0
Mean \pm SD	4.05 \pm 0.85	-7.9 \pm 8.9		100 \pm 119	3.93 \pm 0.87	-1.1 \pm 2.1		3.95 \pm 0.62
								-0.4 \pm 2.7
								0

Definition of abbreviations: CsA = cyclosporine A; DLPC = dialauryolphosphatidylcholine.

* Subjects were exposed to 45 min of continuous CsA and/or DLPC-liposome aerosol administered through a mouthpiece or a face mask attached to an Aerotech II nebulizer at a flow of 10 L/min. Subjects' pulmonary functions were monitored for at least 6 h (see Methods). FEV₁ values returned to starting values by 1 h after exposure and through the 1-wk follow-up. See Table 1 for description of the study.

¹ Change (%) in FEV₁ at the end of aerosol exposure compared with the start. For CsA-DLPC compared with DLPC only, both administered by mouthpiece or compared with CsA-DLPC administered by face mask, $p = 0.041$ or $p = 0.032$, respectively. *t* test, two-tailed.

² I/C, duration of local irritation (I) in the oropharynx and/or cough (C) during and/or after aerosol exposure.

³ Duration of local irritation and/or cough.

ferent from that measured with CsA-DLPC (Part 1) given by mouthpiece ($p = 0.041$; Student's t test, two-tailed). There was no coughing or tracheal irritation reported (Table 2).

Exposure to CsA-DLPC Aerosol Administered by Mouth-only Face Mask (Part 3)

A month after the completion of Part 2, five of the initial 10 volunteers (Table 2), all of whom had reacted previously to CsA-DLPC aerosol, were given another 45-min exposure to CsA-DLPC in the same dosage as in the first part of the study. However, the aerosol was administered through a face mask that permitted breathing only by mouth. The aerosol particle characteristics and CsA concentration were the same as those when the mouthpiece was used (Part 1). As with the administration of DLPC only, there were no alterations in FEV₁ (Table 2) or FVC (not shown) values before or after inhalations (-0.4% ; Table 2). The changes in FEV₁ immediately after exposure were statistically significantly different from those measured with CsA-DLPC (Part 1) given by mouthpiece ($p = 0.032$; Student's t test, two-tailed). There was no coughing or tracheal irritation reported (Table 2).

Regional Deposition

Table 3 shows the calculated total and regional dose of CsA given to the 10 volunteers in a 45-min aerosol exposure. Based on a mean concentration of CsA in the aerosol of 118 $\mu\text{g/L}$, the mean total deposited dosage was estimated to be 13.7 mg in 45 min, with 9.6 mg deposited in Weibel generations 17 to 23 (lungs), 2.3 mg deposited in Weibel generations 0 to 16 (central airways), and 1.8 mg deposited in the mouth. The dosage of DLPC can be calculated by multiplying the CsA values by 7.5, the ratio of DLPC to CsA in the aerosol.

CsA Blood Levels

Blood was drawn from each subject 15 min after the 45-min aerosol exposure by face mask, for measurement of CsA levels (Part 3). All five volunteers had measurable CsA levels ranging from 25 to 140 ng/ml, with a mean of 83 ng/ml (Table 1). Blood was also obtained before and at 24 h after aerosol exposure with the mouthpiece (Part 1). None of these later specimens had measurable levels of CsA (minimum detection level: 25 ng/ml).

Hematology and Blood Chemistry Results

Hematology and blood chemistry analyses were performed on samples obtained at various times in Part 1 (see METHODS).

There were no significant variations in these values at any time in the study (data not shown).

DISCUSSION

This report has described three exposures of 10 volunteers or subgroups thereof to CsA-DLPC liposome aerosols. The liposomes were first inhaled through a plastic mouthpiece of an Aerotech II nebulizer by 10 volunteers. Many of the 10 volunteers experienced intermittent coughing and irritation associated with the exposure. A few days later, nine of the volunteers were given inhalations of DLPC liposome aerosol without CsA, again through a plastic mouthpiece. There was no coughing, irritation, or change in lung function. Five of the 10 volunteers who had had the most severe reactions to the initial exposure were given CsA-DLPC liposome aerosol again, but this time through a face mask that allowed only mouth breathing (mouth-only face mask). There were no reactions to the treatment. That mouth-only breathing through a face mask was unaccompanied by the irritation that occurred when the plastic mouthpiece was used was not due to aerosol characteristics, since both particle size and CsA concentration were similar. It may possibly be explained by the property of the mouthpiece of focusing a jet of aerosol on the back of the throat, where particles will impact and cause irritation. The five subjects who received CsA by face mask noted that they could assume more comfortable positions during exposure, and found the experience to be less stressful.

Except for a transient and self-limited reduction in FEV₁ and FVC values during and immediately after exposure, noted only in the 10 volunteers first treated with CsA-DLPC liposome aerosol given through a plastic mouthpiece (Part 1), there was no spirometric, hematologic, or blood chemical evidence of toxicity. The safety of the treatment is also supported by the finding of lack of toxicity of CsA-DLPC liposome aerosol given to rats for 1 h daily for 28 d in the same concentration used in the present study (10).

Aerosol was administered to the volunteers for 45 min during each of the three experiments in the study. During this time, an average of 13.7 mg of CsA was estimated to be deposited in the lungs (Weibel generations 0 to 23), with 70% of the inhaled dose deposited in the alveolated area of the lungs (Weibel generations 17 to 23). There was thus a sharply targeted deposition in the lungs, with very little CsA deposited above the larynx, where it would be promptly swallowed and would be essentially oral medication. There would also be a slow upward movement of the deposition in Weibel genera-

TABLE 3
ESTIMATED EXPOSURE DOSE AND DEPOSITION OF CYCLOSPORINE A FOLLOWING AEROSOL ADMINISTRATION

Subject	Age (yr)	Weight (kg)	CsA Exposed Dose*			Amount (mg) Deposited in Weibel Generations ¹			
			$\mu\text{g/kg/min}$	$\mu\text{g/kg/45 min}$	mg/45 min	17-23 (P)	0-16 (TB)	Mouth	Total
1	40.3	93.2	12.7	573	53.4	11.7	2.7	2.2	16.6
2	28.6	75.0	12.7	573	43.0	9.4	2.2	1.8	13.4
3	35.2	68.0	12.7	573	39.0	8.5	2.0	1.6	12.1
4	24.0	78.6	12.7	573	45.1	9.9	2.3	1.9	14.0
5	31.2	118.2	12.7	573	67.8	14.8	3.5	2.8	21.1
6	36.1	68.6	12.7	573	39.4	8.6	2.0	1.6	12.3
7	54.1	81.8	12.7	573	46.9	10.3	2.4	1.9	14.6
8	22.3	65.9	12.7	573	37.8	8.3	1.9	1.6	11.8
9	40.1	61.8	12.7	573	35.5	7.8	1.8	1.5	11.0
10	33.9	56.8	12.7	573	32.6	7.1	1.7	1.3	10.1
Mean \pm SD	34.6 \pm 9.2	76.8 \pm 17.9	12.7	573	44.0 \pm 10.3	9.6 \pm 2.3	2.3 \pm 0.5	1.8 \pm 0.4	13.7 \pm 3.2

* Based on 0.108 L-min/kg of body weight (15).

¹ Distribution of deposited dose with mouth-only breathing, based on Vt of 1,000 ml, lungs (P, Weibel generations 17 to 23); 21.9%, tracheobronchial (TB, Weibel generations 0 to 16); 5.1%, mouth, 4.1% (16).

TABLE 4
COMPARISON OF REGIONAL AND TOTAL AEROSOL
DEPOSITION IN LUNGS WITH LARGE TIDAL AND
FUNCTIONAL RESIDUAL VOLUMES AND SMALLER
TIDAL AND FUNCTIONAL RESIDUAL VOLUMES

V _T (ml)	Functional Residual Volume (ml)	Deposition Fraction*			
		Mouth	Weibel Generations		Total
			0-16	17-23	
500	2,500	0.016	0.13	0.16	0.31
1,000	3,300	0.028	0.13	0.19	0.34

* Deposition based on Weibel's lung model of nonhygroscopic particles with an MMAD of 1.6 μ m, GSD of 2.0, and density of 1 gm/ml, and a respiratory frequency of 15/min (C. P. Yu, personal communication).

tions 0 to 16 by mucociliary action, although a portion of the CsA would probably be absorbed before this occurred.

To estimate regional CsA deposition for volunteers with varying pulmonary dimensions, it was assumed that their V_T values and functional residual volumes would be approximately proportional to those of Persons and colleagues' model (16). On that basis it was expected that regional deposition in normal subjects with smaller lung volumes could be calculated with the model. The calculated values shown in Table 4 verify this assumption, in that an aerosol of 1.6 μ m MMAD and with a GSD of 2.0 (nonhygroscopic) deposits almost identical amounts of CsA in the Persons and colleagues' model lung and in lungs with much smaller tidal and functional residual volumes. These data show closely similar patterns for regional deposition in patients with smaller values for tidal and functional residual volumes, that are approximately proportional to those of Persons and colleagues' model. This is the first use of this methodology so far as we know, but the level of agreement was excellent.

If one assumes a weight of 700 gm for an average human lung (right and left), the maximum concentration of CsA in the lungs of the volunteers would be 11.9 mg/700 gm (Table 3), or 17 μ g/gm of lung tissue. Blood levels immediately following treatment averaged 83 ± 42 ng of CsA/ml. This would indicate a CsA concentration in the lungs of about 85-fold greater than the trough concentration in blood recommended for the treatment of lung-transplant rejection (i.e., 200 ng/ml), but with a blood concentration well below the recommended trough concentration of CsA. On the basis of studies in mice in which CsA is substantially cleared from the lungs within 1 to 1.5 h after inhalation in liposomes, blood concentrations would be unlikely to exceed 83 ng/ml with this dose at any time.

The method described here for total and regional CsA dose calculations can be applied to subjects with different lung sizes as long as they are based on individuals with normal lungs. We have done 99m Tc lung scans in patients with severe asthma and found that liposomes labeled with 99m Tc deposited more centrally (Weibel generations 0 to 16) than they would in normal lungs (17). Although, the degree of alteration of lungs in disease will vary widely from time to time and in different pa-

tients, wide diffusion of drug is likely to occur in normal and diseased lungs when substantial concentrations of drug are deposited in the lungs, diminishing the significance of differences in deposition due to disease.

The findings in this report indicate that it is reasonably safe to continue to explore the use of CsA liposome aerosol treatment in subjects with asthma and other pulmonary hypersensitivity diseases.

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